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Remediation of Acenaphthene and Fluoranthene by *Chlorella vulgaris* Beijerinck: FTIR based study

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ABSTRACT
In this study Fourier transform infrared micro-spectroscopy (FTIR) was used to determine degradation of two Polycyclic Aromatic Hydrocarbon-Acenaphthene and Fluoranthene over time in the freshwater microalgae *Chlorella vulgaris* grown in laboratory environmental condition. Microalgae exhibited restricted cell division over the increasing time period and concentration of both the PAHs. FTIR spectra were obtained of the untreated samples and have been referred in the study as zero day. Spectra of treated samples were obtained on 4th day of incubation and on 16th day of incubation. A comparison of FTIR spectra of treated (4th day and 16th day) and untreated sample revealed the presence of new bands pertaining to aliphatic and polycyclic aromatic hydrocarbons including various alcohols, aldehydes, ketones and simple form metabolites. The selected microalgal strain seems to have substantial potential to remediate the hydrocarbon and indicate signs of bioremediation of acenaphthene and fluoranthene.
Keywords: FTIR, *C. vulgaris*, Acenaphthene, Fluoranthene, LC50 determination.

INTRODUCTION

The most well known aquatic pollution caused by petroleum is without doubt pollution due to tanker accidents. Therefore a lot of methods are developed to clean the water from these oil fractions. Another source of pollution in the aquatic environment is hydrocarbon polluted wastewater from industry. Because this is a much more controlled environment more possibilities for degradation are available. An important factor influencing the degradation of hydrocarbons is their availability for chemical and biodegradation. When oil is able to come in contact with sediment it is far less available for biodegradation and becomes persistent. This is one of the reasons for cleaning up the oil as fast as possible [1].

Petroleum-derived compounds, such as polycyclic aromatic hydrocarbons (PAHs) are relatively stable constituents of petroleum, and from the environmental aspect, they are probably the most important analytes because many of these compounds are potential or proven carcinogens [2]. Unfortunately their low aqueous solubility, limited volatility, and recalcitrance towards degradation allows PAHs to accumulate to levels at which they may exert toxic effects upon the environment. Many workers [3, 4] have found that different methods give complementary information for the analysis of petroleum derived compounds in the environment. We had undergone FTIR analytical method for petroleum pollution study:

MATERIALS AND METHODS.

Strain Selection and Incubation

The axenic cultures of microalgae *Chlorella vulgaris* Beijerinck was obtained from the Central Marine and Salt Research Institute, Bhavnagar, India. The growth media selected for microalgae *C. vulgaris* was developed in Zarrouks medium [15] under controlled illumination of 40µEm-2s-1 at 27±1°C in aerobic and static conditions. All inoculations were carried out under aseptic conditions and the cultures were periodically checked for any contamination.

Infrared (IR) absorption spectrometry techniques: Infrared and Raman spectroscopy are the most powerful tools for the identification and characterization of chemical structures. They represent two complementary and versatile techniques to obtain chemicals information in many scientific fields. These techniques were used for the characterization of paper from ancient books and for identification of their degradation products.[5] FTIR Spectroscopy has been widely used to provide the information on range of vibrationally active functional groups (including O-H, N-H, C=O, =C-H, -CH2, -CH3, C-O-C, and >P=O) in biological specimens [6]. Polycyclic aromatic hydrocarbons (PAHs) are now thought to be the most plentiful and widespread class of organic compounds in the universe. Their infrared (IR) signature is associated with many different galactic and extragalactic objects and generally dominates their mid-IR emission [7,8,9,10]. The spectral details of the PAH features vary between different classes of object and spatially within extended objects [11, 12, 13, 14], showing that the details in the emission spectrum depend on, and therefore reflect, the specific PAH molecules present and the conditions within the emission zones. In our present work we had undergone the FTIR based study up to 16days to identify degradation product formed by *C. vulgaris* when treated with acenaphthene and fluoranthene.

LC_{50} values of the organisms for Acenaphthene and Fluoranthene were determined in terms of quantitative estimation of chlorophyll and accordingly, various concentrations of the PAHs were used in all further experiments (Table: I). Stock solution of both the PAHs were prepared in Acetone (Merck made) and added aseptically to the culture medium to the final concentrations indicated for each treatment. Samples (Control untreated and treated) were taken after every four and sixteen days for analysis using Fourier Transform Infrared Spectroscopy (FTIR).

Sample Preparation for Fourier Transform Infrared Spectroscopy (FTIR)

Sample preparation was carried out as described by Naumann et al. [16]. Briefly, known weight of algal samples (1 mg), dried after an interval of four and sixteen days was taken in a smooth agate mortar and mixed thoroughly with 2.5 mg of dry potassium bromide (KBr) using a pestle. The powder was filled in the micro-cup of 2 mm internal diameter to obtain the diffuse reflectance infrared spectrum for replicate samples. All IR spectra were recorded at room temperature (26 ℃ ± 1° C) in the mid infrared range (4000–400 cm^{-1}) using Perkin-Elmer FTIR (Perkin Elmer Model No. 337) Spectrophotometer.

RESULTS AND DISCUSSIONS

We examined the use of FTIR as a sensitive and high throughput means to assess changes in chemical composition of *C. vulgaris* in response to two PAHs. Functional groups present on the cell surface can be identified by FTIR as each group has a unique absorption band [17]. Several other researchers have also used similar FTIR technique for studying changes in the chemical composition in higher plants and algae [18]. The FTIR spectrums of *Chlorella vulgaris* Beijrenick after 4 and 16 days are depicted in table II and III. FTIR spectra of *C. vulgaris* cells showed distinct absorption bands over the wave number range 3460–400 cm^{-1}. The bands were assigned to specific molecular groups on the basis of biochemical standards and published studies [19].

Analysis of C. vulgaris untreated: The FTIR spectrum (Fig. 1) of untreated C. vulgaris (0 day) revealed bands at 3440.73 cm^{-1} indicating hydroxyl stretching in phenols, alcohols; at 2925.43 cm^{-1} signifying -C-H stretching in aliphatic compounds; at 1632.84 cm^{-1} due to the presence of carboxylates; at 779.64 cm indicating the presence of alkyl halides [20].

Analysis of C. vulgaris treated with Acenaphthene: The FTIR spectrum after 4 days of microalgal incubation of *C. vulgaris* with 1.25 ppm, 2.5 ppm and 5.0 ppm Acenaphthene indicated bands at 2925.62 cm^{-1} and 1632.420 cm^{-1} 848.93 cm^{-1} shows the presence of chloro compounds due to C-Cl stretching frequency but with increasing time and concentration disappearance of bands of chloro compound was observed at 4^{th} and 16^{th} day with 2.5ppm and 5.0 ppm respectively. [21]. Band at 1270.56 cm^{-1} was observed which indicates presence of aromatic amines due to C-N stretching frequency, also the band of aromatic compound was observed at 1458.30 cm^{-1} due to presence of Acenaphthene. The band at 848.93 was observed initially, but it completely disappeared after 16^{th} day of treatment which signifies the chloro compounds got disappeared after high dose treatment. A broad peak of naphthalene was characterized at 619.20 cm^{-1} which indicated that Acenaphthene was converted to naphthalene by microalgal degradation. To study protein characterization in *Chlorella vulgaris*, protein spectra characterized by strong peaks 1632 cm^{-1} (amide I) but it disappeared at 16^{th} day in 5 ppm concentration. These bands were due primarily to C=O stretching vibration and a combination of N-H and C-H stretching vibrations in amide complexes [22]. The disappearance of protein from microalgae can be due to toxicity increased with time and concentration. Also the transmittance increased significantly after the treatment with *C. vulgaris*. During microalgal incubation, there was not observed much significant changes.

Analysis of C. vulgaris treated with Fluoranthene: The FTIR spectrum (Fig. 4) of 4 day revealed bands at 2852.45 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 1640.76 cm^{-1} corresponding to primary amides due to N-H stretching [23], at 1458.1cm^{-1} due to C=C in aromatic compound and 1384.63 cm related to CH_3 in aliphatic compounds, elimination of aromatic compound at 1458 cm^{-1} was observed in both 3ppm and 6 ppm at 4^{th} and 16^{th} day. The band at 795.4 cm^{-1} signifying the presence of alkyl halides [24]. The FTIR spectrum recorded after 16 days of microalgal incubation indicated disappearance of primary amides bands in 1.5 ppm was observed. The band of naphthalene was observed at 16^{th} day in 1.5 ppm, 4^{th} and 16^{th} day in 3ppm and 6ppm of Fluoranthene at 619.84 cm^{-1}. The transmittance increased from zero day to 16 day of microalgal treatment indicating the possible reduction in hydrocarbon compounds used by the micro flora as sole sources of carbon and energy. At given environmental conditions, the degree of hydrocarbon biodegradation is mainly affected by the type of hydrocarbons in the contaminant matrix [25]. Of the various petroleum fractions, n-alkanes and branched alkanes of intermediate length (C10–C20) are the preferred substrates to microorganisms and tend to be most readily degradable. Longer chain alkanes (>C20) are hydrophobic solids and are difficult to degrade due to their inherent recalcitrance and their poor water solubility.

CONCLUSION

The FTIR spectra recorded at different time intervals delivered a good indication of hydrocarbon degradability by *C. vulgaris*. The results suggest that the microalgal strain prefer C-H aliphatic and aromatic stretches for degradation of long chain alkanes of PAHs. It is therefore concluded that by using FTIR spectroscopy is very useful tool in performing preliminary tests in order to predict remediation performance so as to select an appropriate approach for clean- up technologies.

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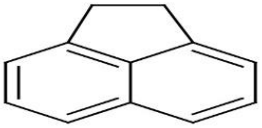
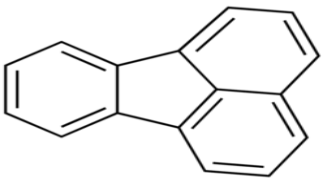
Table I: Represents the PAHs treatments of the test organism for Acenaphthene and Fluoranthene based on their LC_{50} values.

Organism selected for study	Class of compound	Xenobiotic Compound	Structure	Treatments decided based upon LC_{50} (ppm)
C. vulgaris	Polycyclic Aromatic Hydrocarbons	Acenaphthene		1.25
				2.5
				5
		Fluoranthene		1.5
				3
				6

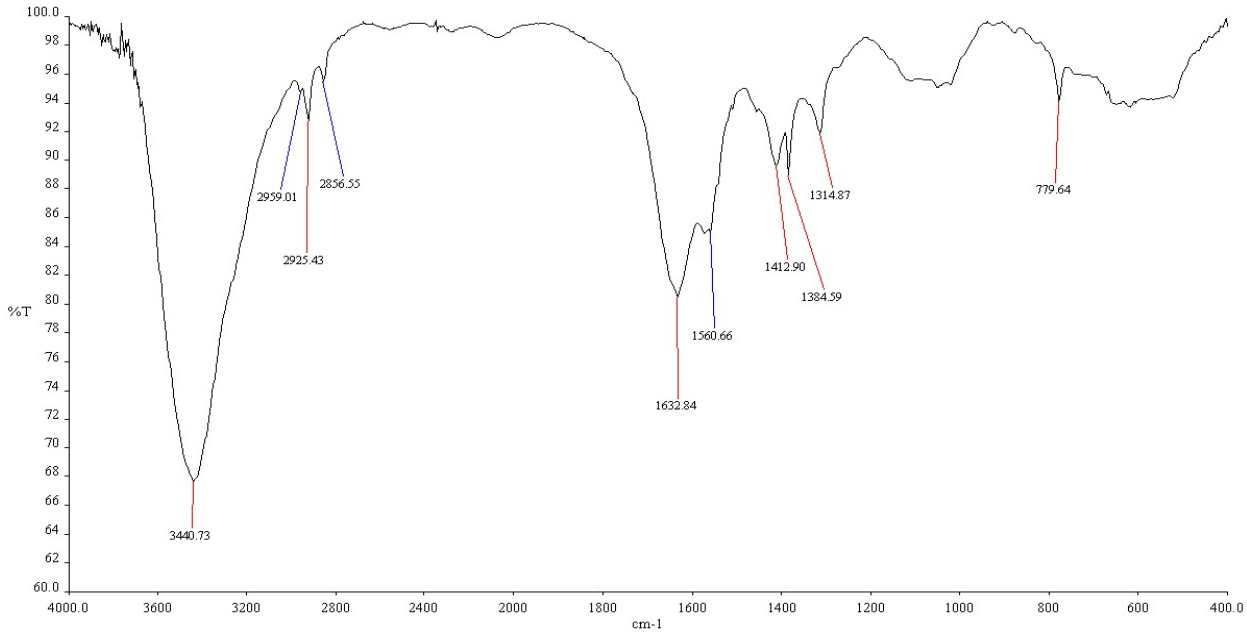


Fig 1: FTIR Spectrum of C. vulgaris untreated (0 Day).

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Table II. The FTIR spectrum of treated C. vulgaris with different concentration of Acenaphthene.
*s-strong, m-medium, w-weak, br-broad, d-disappeared

Sr. No	Range of wave numbers (cm^{-1}) Band assignment	Band position in cm^{-1}											
		Acenaphthene 1.25 ppm				Acenaphthene 2.5 ppm				Acenaphthene 5.0 ppm			
		4th Day		16th Day		4th Day		16th Day		4th Day		16th Day	
1	645-615 naphthalene	619.20	br	618.00	br	620.88	br	619.84	br	622.73	w	600.40	s
2	790-750 –(CH_2)n– in hydrocarbons	779.81	m	780.07	w	778.03	w	778.03	w	780.07	w	780.07	w
3	850-550 C–Cl in Chloro compound	848.93	w	849.21	m	847.50	w	--	d	--	d	--	d
4	1280-1180 C–N in aromatic amines	1270.56	w	1270.49	w	1270.49	w	1273.91	w	1273.91	w	1270.49	w
5	1390-1370 CH_3 in aliphatic compounds	1384.62	m	1384.57	s	1384.59	m	1384.58	s	1384.68	m	1384.73	s
6	1465-1400 C=C in aromatic compound	-	-	1458.30	m	1456.44	w	1454.40	m	1456.44	w	1457.32	m
7	1640-1580 NH_3 + in amino acids	1632.42	s	1632.38	s	1633.37	s	1630.94	s	1629.18	s	--	d
8	2990-3100 =CH in aromatic and unsaturated hydrocarbons	2925.62	w	2926.22	w	2926.22	w	2925.26	m	2925.02	m	2926.02	w
9	3450-3250 –OH in alcohols and phenols	3441.53	br	3460.25	br	3443.48	br	3440.20	br	3440.44	br	3459.59	br

Table III: The FTIR spectrum of treated C. vulgaris with different concentration of Fluoranthene.
*s-strong, m-medium, w-weak, br-broad, d-disappeared.

Sr. No	Range of wave numbers (cm^{-1})	Band position in cm^{-1}											
		Fluoranthene 1.5 ppm				Fluoranthene 3 ppm				Fluoranthene 6 ppm			
		4th Day		16th Day		4th Day		16th Day		4th Day		16th Day	
1	600-465 G–I in iodo compounds	595.8	br	--	d	--	d	--	d	--	d	--	d
2	645-615 naphthalene	--	--	619.84	w	620.38	w	619.19	br	621.95	w	620.42	w
3	780-720 –(CH_2)n– in hydrocarbons	779.91	s	780.07	w	779.65	w	778.03	w	779.44	w	780.07	w
4	895-850 1,2,4-trisubst benzenes	865.90	w	865.90	m	865.90	w	--	d	865.39	w	865.90	w
5	1280-1180 G–N in aromatic amines	1268.45	w	1270.76	s	--	d	1270.74	s	--	d	1270.49	w
6	1390-1370 CH_3 in aliphatic compounds	1384.63	s	1384.76	s	1384.64	w	1384.41	w	1384.59	w	1384.37	w
7	1465-1400 C=C in aromatic compound	1458.11	br	1454.94	s	--	d	--	d	--	d	--	d
8	1640-1580 NH_3 + in amino acids	1640.76	m	--	d	1632.72	m	1634.81	w	1632.89	m	1630.21	w
9	2990-2850 – CH_3 and – CH_2 in aliphatic compound	2852.45	w	--	d	2856.55	w	2856.55	w	2856.55	w	2856.55	w
10	3500-3200 –OH in alcohols and phenols	3459.14	br	3428.76	br	3441.99	br	3440.35	br	3438.14	br	3439.94	br